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UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

GEN-PROBE INCORPORATED, Plaintiff, v. VYSIS, INC., Defendant.	No. 99-CV-2668H AJB **SUPPLEMENTAL EXPERT REPORT OF FRED R. KRAMER**

1. I am a member and chairman of the Department of Molecular Genetics, The Public Health Research Institute in New York, New York and am a research professor of microbiology and cell biology at the New York University School of Medicine. I provide the following opinions as a supplement to my earlier report concerning the lack of enablement of United States Patent No. 5,750,338 ("the '338 patent").

SUMMARY OF MY OPINIONS

2. As set forth in the following paragraphs, I conclude that the disclosure of the '338

patent, in particular, the disclosure of Example 7, failed to teach one of ordinary skill in the art to achieve either linear or exponential amplification of a target nucleic acid using Qß replicase. In particular, I believe that, as of the filing date of December 21, 1987, one of ordinary skill in the art could not have used the disclosures in the specification of the '338 patent relating to Qß replicase, including Example 7, to amplify heterologous RNA (in other words, RNA that is not bacteriophage Qß genomic RNA or an RNA structurally related to it). The reasons supporting this opinion are set forth in the following paragraphs.

EDUCATION AND EXPERIENCE

3. I began my training and experience relevant to my opinion in this case at the University of Michigan where I received a B.S. (with honors) in Zoology in 1964. I received a Ph.D. from The Rockefeller University in 1969 and did my postdoctoral training at Columbia University from 1969 to 1972 under Dr. Sol Spiegelman. I was employed in various scientific positions from 1969 to 1986 in the Department of Genetics and Development and the Institute of Cancer Research, College of Physicians and Surgeons at Columbia University, including as a Fellow of the American Cancer Society from 1969 to 1971, a Research Associate from 1971 to 1972, an Instructor from 1972 to 1973, an Assistant Professor from 1973 to 1980, a Senior Research Associate from 1980 to 1983, and a Research Scientist from 1983 to 1986. A true and correct copy of my resume is attached to this declaration as Exhibit "A".

4. Example 7 of the '338 patent purports to make use of the enzyme Qß replicase to exponentially amplify target polynucleotides. I am familiar with the use of the enzyme Qß replicase in amplification methods because of my own extensive research in this area. Beginning in 1969, while doing my postdoctoral training, I worked with Dr. Spiegelman on sequencing the nucleotides of replicating RNA molecules and the study of Qß replicase. By 1983, my work demonstrated that one could insert heterologous oligonucleotides at an appropriate site within a naturally occurring Qß template RNA, and the resulting "recombinant RNAs" could be amplified exponentially by incubation with Qß replicase. By 1992, my laboratory demonstrated that one could amplify recombinant mRNAs exponentially in this manner.

5. I am a co-inventor on several United States patents in this field. The list of those

patents is found in Exhibit "A" to this declaration. In addition, I have stayed abreast of the general technology of amplification by regularly reviewing scientific literature and attending scientific conferences. The conclusions I provide in the following declaration are based on my experience and understanding of the reactions involved in Qß replicase amplification and nucleic acid synthesis in general.

THE DISCLOSURES OF THE '338 PATENT

6. The '338 patent describes methods of detecting nucleic acid sequences. The methods described in the '338 patent use the capture of polynucleotide sequences on a solid phase support and non-specific amplification of the captured polynucleotide. I have been informed that the filing date of the first patent application that discloses this combination of steps and from which the '338 patent claims priority is December 21, 1987 (the "filing date").

7. I understand that the question of enablement of the '338 patent must consider the level of skill in the technology relevant to the '338 patent. I consider the level of ordinary skill in the art of molecular biology at the filing date of the '338 patent application to have been that of an individual with a Ph.D. in the biological sciences and two years of postdoctoral experience. Such experience would have allowed the individual to develop the skills of a molecular biologist using the techniques of DNA and RNA isolation and characterization, cDNA synthesis, cloning, liquid and solid phase hybridization (including knowledge of the conditions influencing hybrid formation and stability), affinity chromatography, isotopic and non-isotopic labeling methods, DNA sequencing methods, and nucleic acid amplification, such as by using nucleic acid polymerases.

8. Example 7 of the '338 patent describes non-specific amplification using an RNA polymerase known as Qß replicase. Qß replicase is an enzyme comprised of four polypeptide chains, one of which is encoded in the genome of a viral organism known as bacteriophage Qß. The other three polypeptides are encoded in the genome of the bacterium *Escherichia coli*, which Qß infects. The enzyme has RNA-directed RNA polymerase activity and is isolated from *E. coli* infected with bacteriophage Qß or from bacteria in which the viral gene has been cloned

9. As of 1987, Qß replicase was known to copy *in vivo* and *in vitro* only Qß genomic RNA and smaller RNAs generated in Qß-infected *E. coli* that are related to Qß RNA. This

extraordinary template specificity enables Qß replicase to distinguish Qß RNA from the vast number of different RNA molecules that are present in *E. coli*. Thus, Qß replicase does not copy other nucleic acids and, consequently, the viral RNA is efficiently replicated after infection.

10. The only disclosure provided in the '338 patent to purportedly teach how to use Qß replicase to effect amplification of a target nucleic acid is an isolated reference to an article published in 1980 by Thomas Blumenthal entitled, "Qß Replicase Template Specificity: Different Templates Require Different GTP Concentrations for Initiation," Proc. Natl. Acad. Sci. U.S.A. 77, 2601-2605. Example 7 of the '338 patent cites the Blumenthal paper as the sole description of a technique for exponentially replicating both messenger RNA ("mRNA") and ribosomal RNA ("rRNA") non-specifically using Qß replicase.

11. As generally understood by those skilled in the art, "exponential amplification" is an amplification technique .in which the replication product is a template for amplification. Although Example 7 does not mention "linear" amplification, in contrast with exponential amplification, linear amplification describes a technique wherein multiple copies of a target nucleic acid are generated only from a basic template such that each "cycle" of amplification only results in a linear increase in amplification product. As set forth in the following testimony, I believe that the Blumenthal paper neither purports to nor provides a sufficient disclosure to teach how to use Qß replicase to perform *either* linear amplification or the claimed exponential amplification.

BLUMENTHAL'S DISCLOSURE

12. Blumenthal's paper purports to describe a study of conditions for the "initiation" of synthesis of complementary copies of different target RNA templates as measured by production of acid-insoluble radioactivity. In his paper, Blumenthal reported experiments wherein he merely attempted to *initiate* transcription of three synthetic RNAs and two naturally-occurring heterologous RNAs (bacteriophage f2 RNA and rRNA). However, the Blumenthal paper does not show, nor claim to show, that product RNA representative of the target was actually made by the reaction.

13. In his experiments, Blumenthal varied the concentration of GTP, a nucleotide that is

always used by Qß replicase to initiate transcription, and further changed the amount of manganese and/or salt ("ionic strength") in the reaction. Using radioactive labels, Blumenthal also measured the amount of transcription initiation and the product lengths for the three synthetic RNA target templates. He observed that the amount of initiation product and resulting product lengths varied with each target.

14. As noted above, Blumenthal never fully characterized the results he obtained from his experiments. All that is apparent from his published data is that he initiated transcription of "something" that incorporated labeled nucleotides in the presence of three synthetic and two naturally occurring RNA molecules. Furthermore, it is apparent that the amount of incorporation of that labeled nucleotide was affected by changing the reaction conditions. In particular, Blumenthal reported that the GTP requirement for initiation of synthesis was different for each of the five target RNA templates tested and was further changed by the amount of manganese and/or salt ("ionic strength") in the reaction. However, because Blumenthal did not characterize the actual products of his experiments, it is impossible from the data presented to know whether or not significant amounts of complementary copies of the target RNAs were made. It is also impossible to determine if the products were truly representative of the "target" templates used or merely consisted of fragments of the sequences present, or, still further, were only made up of fragments copied from a specific region of the target RNA molecules.

BLUMENTHAL DOESN'T ENABLE LINEAR "AMPLIFICATION" USING Qß REPLICASE.

15. Thus, even assuming that Blumenthal's experiments actually resulted in the production of full-length complementary transcript copies of the target nucleic acids, Blumenthal's results showed that the reaction conditions for each different target differed appreciably and, most importantly, unpredictably for each different target. As such, in December 1987, in my opinion, a mere reference to Blumenthal's paper would not provide sufficient detail to enable skilled scientists to prepare an initial transcript of any given target nucleic acid using Qß replicase without a significant amount of experimentation for each target, if indeed, such transcription could be achieved at all. In other words, that Blumenthal may have observed the *initiation* of transcription at some level with a limited number of target RNAs under various and unpredictable conditions

would not allow those skilled in the art to determine if the type of synthesis necessary to amplify nucleic acids *in vitro* could be achieved using any of the conditions set forth in the Blumenthal paper.

16. Moreover, a fundamentally more significant problem in the application of Blumenthal's work to any claimed technique for "amplification" of a target nucleic acid with Qß replicase exists in the fact that, even if all the necessary reaction conditions were predictably solved for a given target, Blumenthal's technique, at best, would still not "amplify" the target nucleic acid using Qß replicase. As noted above, Blumenthal's study only attempted to obtain a single, complementary transcript copy of each target nucleic acid. Upon the conclusion of the reaction described in Blumenthal, the complementary transcript copy of the target RNA remains firmly hydrogen-bonded to the target; thus rendering both the target and its complementary copy unavailable for further copying by Qß. Accordingly, even if a complementary transcript copy were successful synthesized, an inherent aspect of the technique used by Blumenthal would resulted in the *termination* of the reaction upon creation of that single complementary transcript. The entire process would result in the creation of only one complementary nucleic acid for each original target molecule. In my opinion, the creation of a single complementary copy per target molecule would not be considered to be "amplification" of a target polynucleotide. As such, for this further reason, Blumenthal does not teach or enable a method of linear "amplification" of a heterologous target nucleic acid using Qß replicase.

BLUMENTHAL DOESN'T ENABLE EXPONENTIAL "AMPLIFICATION" USING Qß REPLICASE.

17. Example 7 of the '338 patent expressly claims to describe a method of exponential amplification of target nucleic acids using the Qß replicase enzyme. In my opinion, Example 7 also does not teach how to use Qß replicase to amplify exponentially a target nucleic acid either.

18. For years prior to 1987, many investigators desired to use Qß replicase to catalyze *in vitro* the exponential synthesis of heterologous RNAs. By 1987, scientists had devised a number of schemes in effort to circumvent the extraordinary specificity of Qß replicase. These strategies were tried with a wide range of heterologous templates, including rRNAs, viral RNAs, and eukaryotic mRNAs. In all cases, the amount of RNA synthesized never exceeded the original

amount of template RNA and the products only consisted of complementary strands that remained hybridized to the template strand. By 1983, my laboratory had shown that one could insert heterologous oligonucleotides at an appropriate site within a naturally occurring Qß template RNA, and the resulting "recombinant RNAs" could be amplified exponentially by incubation with Qß replicase. Ultimately, in a paper we published in 1992, we showed that recombinant RNAs could be amplified exponentially in this manner.

19. In order to use the technique that we discovered and published for exponential amplification of recombinant RNAs for application with heterologous target RNAs, one needed to be able to create recombinant RNAs from heterologous RNAs. Yet, in 1987, no one had any idea how to do that. Thus, in 1987, the disclosure of Example 7 of the '338 patent would not enable one of ordinary skill in the art to use Qß replicase to exponentially amplify heterologous RNAs.

20. A method using Qß replicase to amplify any heterologous RNA molecule efficiently and conveniently in order to produce more RNA products would be extremely valuable. Yet, to this day, no such method is known to the art. In my opinion, if the disclosure in this patent had enabled such a method, it would be of far greater value than the target capture methods described therein.

21. I have been informed that the inventors of the '338 patent did not attempt to actually practice the method described in Example 7. In my opinion, the inventors' failure to attempt to reduce Example 7 to practice provides further support for my view that Example 7 does not teach a means of exponentially amplifying heterologous RNAs using Qß replicase. Similarly, the fact that no one else in the ensuing 15 years has succeeded in developing the claimed technique provides further support as well.

CONCLUSIONS

22. In summary, as of the filing date of the '338 patent, I believe that the disclosure in Example 7 of the '338 patent would not enable one skilled in the art to achieve either linear or exponential target amplification using Qß replicase.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

Dated: October 9, 2001

Fred Russell Kramer

Fred R. Kramer